Original Article

Mast cells and angiogenesis in malignant and premalignant oral lesions: An immunohistochemical study

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Abstract

Introduction and Objectives: Angiogenesis is a complex event facilitated by angiogenic factors released from neoplastic and host immune cells. Among host immune cells, mast cells (MCs) may have greater significance in tumor progression through angiogenesis. The objectives of the study were to evaluate and correlate mast cell density (MCD) and microvessel density (MVD) in normal gingival tissue, leukoplakia with and without dysplasia and oral squamous cell carcinoma (OSCC) cases.

Materials and Methods: Among eighty selected cases, twenty were of normal gingiva, twenty each of leukoplakia without and with dysplasia and twenty of OSCC. The slides were stained with CD34 and counterstained with 0.1% toluidine blue, followed by quantification of MCD and MVD per high-power field (×40) using Image-Pro Express software.

Statistical Analysis: Chi-square test and correlation coefficient were used for statistical analysis.

Observation and Results: A statistically significant difference in the values of MVD and MCD between normal gingival tissue, leukoplakia with and without dysplasia and OSCC ($P = 0.000$) was observed. MVD and MCD showed a positive correlation between the study groups.

Conclusion: MVD and MCD increased significantly in cases of OSCC as compared to leukoplakia with and without dysplasia and normal gingival tissue. It was concluded that MCs may play a significant role in angiogenesis by releasing pro-angiogenic and angiogenic factors which may in turn favor the progression of premalignant lesion to a malignant one.

Keywords: Angiogenesis, CD34, mast cell density, mast cells, microvessel density, toluidine blue

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INTRODUCTION

Mast cells (MCs) were first described by Paul Ehrlich in his 1878 doctoral thesis due to their unique staining characteristics and large granules.[1] Shortly after its discovery, MC's tendency to concentrate around blood vessels in inflammatory and neoplastic foci was identified. It was later demonstrated that they accumulate around tumors before the onset of tumor-associated angiogenesis.[2]

MCs are also a prime source of angiogenic factors. Under physiological conditions, they are particularly prominent in proximity of capillaries and lymphatic channels and also appear at the edges of invasive tumors where they facilitate

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angiogenesis by releasing preformed mediators or by triggering proteolytic release of extracellular matrix-bound angiogenic compound. They are also key host cells in tumor infiltrate with important consequence for tumor cell fate.[3]

Angiogenesis, the growth of new blood vessels from preexisting ones, is an essential phenotype of tumor formation.[4] Accumulation of MCs around tumor margins and the subsequent release of angiogenic factors possibly represent a tumor–host interaction which favors tumor progression.[5]

Stains which identify angiogenesis and MCs include CD34 and toluidine blue, respectively. Present in hematopoietic progenitor cells and endothelial cells, CD34 is an antigen composed of glycoprotein, making it more resistant to formalin fixation and is thus studied as a marker for vascular tumors. Anti-CD34 antibody is a highly sensitive marker for endothelial cell differentiation, which stains neoplastic endothelium a deeper shade than normal endothelium.[6] It has equal staining intensity for small and large blood vessels.[7] In addition, toluidine blue reveals MCs as large, purple, oval and highly granulated cells.[5]

MATERIALS AND METHODS

This retrospective study was conducted on tissue sections obtained from diagnosed cases of leukoplakia and oral squamous cell carcinoma (OSCC), retrieved from archives of the department.

The study group comprised a total of eighty cases, of which twenty cases were normal gingival tissue, twenty each of leukoplakia with and without dysplasia and twenty of OSCC.

Inclusion criteria

1. Normal gingival tissue obtained from patients undergoing tooth extraction for orthodontic purposes
2. All oral leukoplakia cases with and without dysplasia
3. Incisional and excisional biopsies of primary OSCC.

Informed consent was obtained from patients for normal gingival tissues.

Exclusion criteria

1. Secondary and metastatic OSCC cases and other white lesions of the oral cavity
2. Oral mucosa biopsy of patients with signs of inflammatory gingival and periodontal disease.

Monoclonal (1A4) mouse anti-human CD34 antibody (BioGenex) and Super Sensitive™ Polymer-HRP IHC Detection System HRP/DAB (BioGenex) were used for the study. Counterstaining was done with 0.1% toluidine blue (slides were quickly dipped 15 times in toluidine blue solution and washed in tap water to remove the excess chromogen. The slides were quickly dipped once in a solution containing 70% alcohol and 0.5% HCl). Presence of brown-colored precipitate at the site of target antigen indicated positive immunoreactivity. The overall slide background was clear without any extraneous deposits.

The microvessel density (MVD) and mast cell density (MCD) quantification was performed with a binocular light microscope under high-power magnification (×40) in ten consecutive high-power fields (×40) using Image-Pro Express software (Fiji is developed by contributors around the world, and funded from various sources. It is maintained by Curtis Rueden and the ImageJ development team at the Laboratory for Optical and Computational Instrumentation (LOCI) at the University of Wisconsin-Madison). The total count was divided by 10 (number of fields studied) to obtain the average MVD and MCD.

Statistical analysis

Chi-square (χ^2) test was calculated to test the significance of MVD and MCD between the study groups, and correlation coefficient (r) was calculated to determine the interdependency of MVD and MCD.

The hypotheses assumed for χ^2 testing are as follows:

- H_0 (null hypothesis): There is no association of MCs in angiogenesis and angiogenesis in progression from premalignant to malignant lesion
- H_1 (alternative hypothesis): There is an association between MCs in angiogenesis and angiogenesis in progression from premalignant to malignant lesion.
- The hypotheses for testing correlation (r) are stated below:
  - H_0 (null hypothesis): The hypothesis assumed is that if the r value is 0, then there is no association between the groups
  - H_1 (alternative hypothesis): The alternate hypothesis to be taken is that if the r value is $\neq 0$, there is association between the groups.

RESULTS

The study samples were categorized into four groups as Group 1 (normal gingival tissue), Group 2 (leukoplakia without dysplasia), Group 3 (leukoplakia with dysplasia) and Group 4 (OSCC). The tissue sections were stained with CD34 antibody and counterstained with 0.1% toluidine blue. MVD and MCD were quantified in 10 high-power field for each of the study group, following which the total and average values were calculated. Statistical tools were applied to the obtained values.

A statistically significant difference was observed in the χ^2 values of MVD and MCD when compared between the study groups as shown in Tables 1 and 2. The χ^2 test showed MVD to be significantly higher when compared between Groups 3 and 4 than between Groups 2 and 3 and Groups 1 and 2 ($P = 0.000$) [Tables 3-5]. Similarly, the χ^2 value of MCD was significantly higher when compared between Groups 3 and 4 than between Groups 2 and 3 and Groups 1 and 2 ($P = 0.000$) [Tables 6-8]. MVD and MCD were positively correlated ($P = 0.000$) as shown in Tables 9 and 10.

After comparing the χ^2 values of the different groups for 19 degrees of freedom, the null hypothesis was rejected at $P < 0.01$. Therefore, we conclude that there is a significant association between all the groups.

Correlation coefficient (r) is calculated to be 0.109404487. "r" value can range from −1 to +1; a positive r value indicates an increase of one variable when the other variable increases, i.e., the values are directly proportional. Hence, it is concluded that there is an association between MVD and MCD. This implies that the null hypothesis is invalid.

A significant difference was also observed in the average values of MVD [Table 11] between the study groups [Figure 1].

A significant difference was observed in the average values of MCD [Table 12] between the study groups [Figure 2].

DISCUSSION

First described by Paul Ehrlich in his doctoral thesis, MCs are bone marrow-derived tissue-homing leukocytes.[8] They tend to concentrate around blood vessels in inflammatory and neoplastic foci and later accumulate near tumors

Table 1: Microvessel density

Group	χ^2	*P*
Between Groups 1 and 2	215.4486577	0.000
Between Groups 2 and 3	236.8546642	0.000
Between Groups 3 and 4	250.7309413	0.000

Table 2: Mast cell density

Group	χ^2	*P*
Between Groups 1 and 2	220.478072	0.000
Between Groups 2 and 3	270.2411918	0.000
Between Groups 3 and 4	805.1321712	0.000

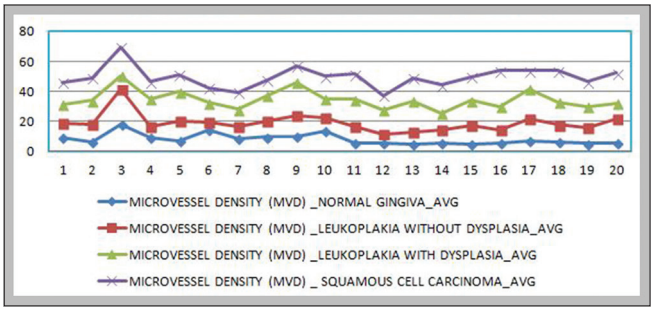

Figure 1: Comparison of microvessel density between the study groups

Table 3: Chi-square value of microvessel density between Groups 1 and 2

Case number	Chi-square method							
	Total observed values			Total expected values			$(OBS-EXP)^2/(EXP)$	
	Group 1	Group 2	Grand total	Group 1	Group 2	Grand total		
1	90	97	187	78.10029	108.8997	187	1.81309376	1.3003078
2	61	121	182	76.01205	105.988	182	2.96481296	2.126293467
3	177	235	412	172.0712	239.9288	412	0.141179007	0.101250232
4	95	73	168	70.16496	97.83504	168	8.790412478	6.304275136
5	70	133	203	84.78267	118.2173	203	2.57749861	1.848520811
6	144	51	195	81.44148	113.5585	195	48.05375558	34.46301266
7	85	81	166	69.32967	96.67033	166	3.541908265	2.540172519
8	96	107	203	84.78267	118.2173	203	1.484131098	1.064383589
9	98	143	241	100.6533	140.3467	241	0.069943715	0.050161972
10	133	91	224	93.55329	130.4467	224	16.63269451	11.92857362
11	52	113	165	68.91202	96.08798	165	4.150457155	2.976609335
12	53	64	117	48.86489	68.13511	117	0.349927491	0.250959689
13	45	84	129	53.87667	75.12333	129	1.462511671	1.048878649
14	54	87	141	58.88845	82.11155	141	0.40580057	0.291030535
15	48	126	174	72.67086	101.3291	174	8.375450343	6.006674144
16	56	86	142	59.3061	82.6939	142	0.184303202	0.132177881
17	68	150	218	91.04739	126.9526	218	5.834130679	4.184100015
18	64	112	176	73.50615	102.4938	176	1.229379434	0.881681743
19	51	110	161	67.24142	93.75858	161	3.922936947	2.813437244
20	55	160	215	89.79445	125.2056	215	13.48250013	9.669329006
Total	1595	2224	3819	1595	2224		χ^2=215.4486577 *P*=0.000	

Table 4: Chi-square value of microvessel density between Groups 2 and 3

Chi-square method								
Case number	Total observed values			Total expected values			$(OBS-EXP)^2/(EXP)$	
	Group 2	Group 3	Grand total	Group 2	Group 3	Grand total		
1	97	124	221	92.6492	128.3508	221	0.204313375	0.147482293
2	121	157	278	116.5451	161.4549	278	0.170283569	0.122918097
3	235	90	325	136.2488	188.7512	325	71.57342757	51.66481756
4	73	181	254	106.4837	147.5163	254	10.52891534	7.600229704
5	133	195	328	137.5065	190.4935	328	0.14769172	0.106610317
6	51	129	180	75.46089	104.5391	180	7.929073906	5.723550915
7	81	115	196	82.16852	113.8315	196	0.016617551	0.011995272
8	107	170	277	116.1259	160.8741	277	0.717173197	0.517686852
9	143	219	362	151.7602	210.2398	362	0.505676389	0.365019243
10	91	125	216	90.55306	125.4469	216	0.002205917	0.001592327
11	113	182	295	123.672	171.328	295	0.920917734	0.664758533
12	64	158	222	93.06843	128.9316	222	9.079055348	6.553657609
13	84	207	291	121.9951	169.0049	291	11.83348805	8.541927106
14	87	112	199	83.4262	115.5738	199	0.153093801	0.110509774
15	126	164	290	121.5759	168.4241	290	0.160993377	0.116212032
16	86	157	243	101.8722	141.1278	243	2.472967276	1.785095496
17	150	199	349	146.3103	202.6897	349	0.093049398	0.067167109
18	112	153	265	111.0952	153.9048	265	0.007369134	0.005319362
19	110	137	247	103.5491	143.4509	247	0.401877461	0.290092656
20	160	107	267	111.9336	155.0664	267	20.64056961	14.89926219
Total	2224	3081	5305	2224	3081		χ^2=236.8546642	
							P=0.000	

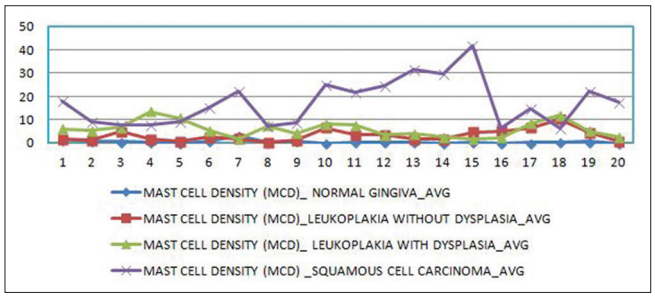

Figure 2: Comparison of mast cell density between the study groups

before the onset of tumor-associated angiogenesis.[2] They also play an important function in the regulation of physiological and pathological neovascularization, mostly on the basis of histological observations.[9] Therefore, this study was undertaken to evaluate and correlate MCD and MVD in normal gingival tissue, leukoplakia with and without dysplasia and OSCC.

A correlational study of MVD and MCD by Sharma *et al.* revealed a linear increase in MVD and MCD, suggesting a positive correlation.[10] Further, Michailidou *et al.* observed a significant increase in MVD and MCD between the cases of normal mucosa, leukoplakia without dysplasia, leukoplakia with mild, moderate or severe dysplasia and OSCC. They concluded that an angiogenic switch seemed to be turned on in the early stages of epithelial malignant transformation.[5]

A study by Mohtasham *et al.*, on MCD and angiogenesis in oral dysplastic epithelium and low- and high-grade OSCC using CD34 and counterstaining with Meyer's hematoxylin, found a significant correlation between MC count and MVD in agreement with the concept that MCs promote tumor progression through upregulation of angiogenesis. MC count and the degree of angiogenesis can be potentially used as an indicator of evolution of squamous cell carcinoma from epithelial dysplasia.[11]

The present study was conducted in agreement with previous studies conducted by Michailidou *et al.*, Mohtashan *et al.* and Sharma *et al.*

MCs are a prime source of angiogenic factors. Under physiological conditions, they are particularly prominent near capillaries and lymphatic channels. In many inflammatory disorders characterized by profound vascular remodeling, the infiltrate exhibits numerous MCs which show structural features of degranulating elements. In various tumor models, MCs appear at the edges of invasive tumors to facilitate angiogenesis by releasing preformed mediators or by triggering proteolytic release of extracellular matrix-bound angiogenic compounds.[3] Angiogenesis refers to the formation of new blood vessels from preexisting vascular structures, i.e., capillaries and postcapillary venules. It is a critical process in tumor progression as vascular networks produced by the host are essential to allow neoplastic cell populations to form a clinically observable tumor.[3]

In 1971, Dr. Judah Folkman published a landmark paper in the New England Journal of Medicine, hypothesizing

that solid tumor promoted angiogenesis in the tumor microenvironment by secreting pro-angiogenic factors.[12] Angiogenic factors are produced by tumor cells and accessory host cells such as macrophages, MCs and lymphocytes which may be attracted to the tumor.[13] Angiogenesis enhances entry of tumor cells into the circulation by providing an increased density of immature, highly permeable blood vessels which have little basement membrane and fewer intercellular junctional complexes than normal mature vessels.[13]

Associated with metastasis, intratumoral blood vessels are known to play an important role in cancer growth by supplying oxygen, nutrients and excreting metabolic products.[14] In 1945, Algire *et al*. first reported active neovascularization by the host to neoplastic tissues. Later, Folkman conducted a series of studies on cancer growth and neovascularization, and demonstrated that blood vessels in the host underwent angiogenesis and developed into intratumoral vessels that were closely related to tumor growth.[14] Williams *et al*. demonstrated a correlation between recurrence of OSCC and blood vessel count.[14] Moriyama *et al*. reported a correlation between metastasis and vessel density at the tip of infiltration when measured using CD31.[14]

Gruber *et al*. account that angiogenic factors including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and platelet-derived growth factor-AB stimulate MC migration. MCs might migrate into hypoxic areas chemotactically as a result of angiogenic factors released from cancer cells. Once in these hypoxic areas, MCs might produce angiogenic products that stimulate infiltration of more MCs.[15]

To grow to 2 mm^3 or more, solid tumors require oxygen. This necessitates the formation of new microvasculature. Angiogenesis occurs due to imbalance of positive and negative angiogenic factors produced by tumor and host cells. Among the host cells, MCs produce and release considerable quantities of pro-angiogenic and angiogenic factors. The factors such as histamine, heparin, chymase, bFGF and VEGF promote the migration or proliferation of endothelial cells.[5]

To measure angiogenic activity in this study, a Pan-endothelial marker (CD34) was used to stain microvessels; counterstaining was performed with 0.1% toluidine blue to observe the possible angiogenic activity of MCs at the same optical field as microvessels [Figures 3-6].

MCs surround various tumors and are sometimes detected in large numbers before the occurrence of neovascularization in some malignancies. MC-deficient mice exhibit less tumor angiogenesis when compared to mice with normal

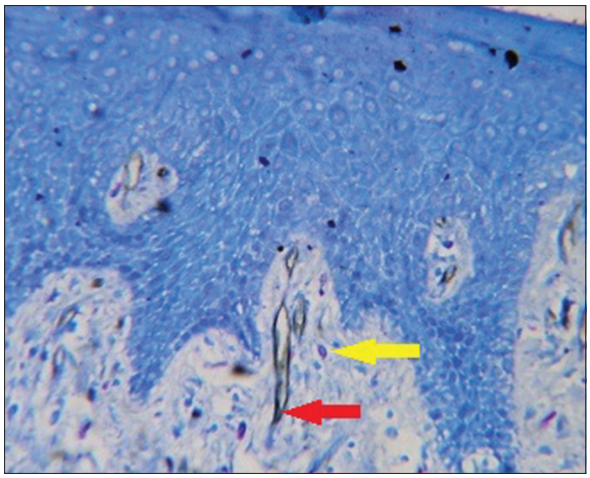

Figure 3: Photomicrograph of normal gingival tissue (red arrow - microvessel, yellow arrow - mast cell)

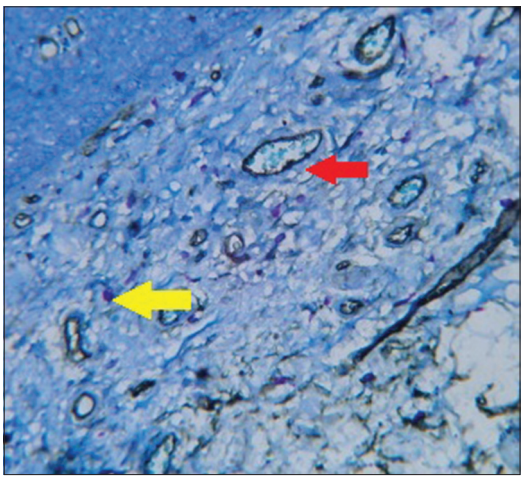

Figure 4: Photomicrograph of leukoplakia without dysplasia (red arrow - microvessel, yellow arrow - mast cell)

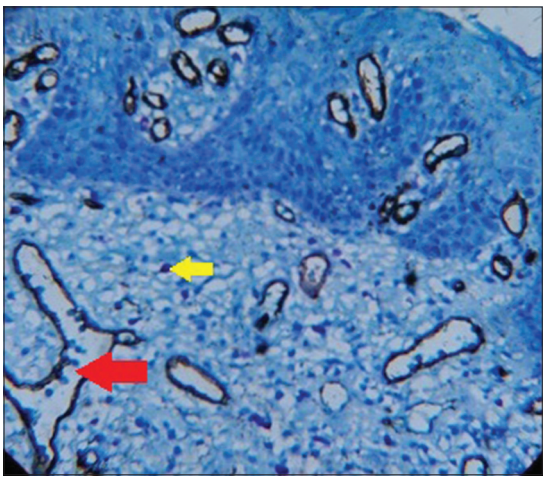

Figure 5: Photomicrograph of leukoplakia with dysplasia (red arrow - microvessel, yellow arrow - mast cell)

MC numbers. Moreover, they were found to induce neovascularization through carcinogenesis of squamous cells.[16]

Table 5: Chi-square value of microvessel density between Groups 3 and 4

Chi-square method								
Case number	Total observed values			Total expected values			$(OBS-EXP)^2/(EXP)$	
	Group 3	Group 4	Grand total	Group 3	Group 4	Grand total		
1	124	148	272	137.6531	134.3469	272	1.354178215	1.387503519
2	157	152	309	156.378	152.622	309	0.002474377	0.002535269
3	90	193	283	143.2199	139.7801	283	19.7763111	20.26299118
4	181	116	297	150.305	146.695	297	6.268462439	6.422724567
5	195	113	308	155.8719	152.1281	308	9.82223255	10.06395028
6	129	98	227	114.8796	112.1204	227	1.735605972	1.778317925
7	115	114	229	115.8918	113.1082	229	0.006861797	0.007030661
8	170	103	273	138.1592	134.8408	273	7.338193835	7.518781246
9	219	111	330	167.0056	162.9944	330	16.18759768	16.58596224
10	125	153	278	140.6896	137.3104	278	1.749682721	1.792741091
11	182	170	352	178.1393	173.8607	352	0.083670921	0.08573
12	158	99	257	130.0619	126.9381	257	6.001264618	6.14895121
13	207	152	359	181.6818	177.3182	359	3.528198519	3.615024821
14	112	189	301	152.3293	148.6707	301	10.67723009	10.93998866
15	164	160	324	163.9691	160.0309	324	5.81573E-06	5.95885E-06
16	157	238	395	199.9006	195.0994	395	9.206892487	9.433467161
17	199	122	321	162.4509	158.5491	321	8.223024734	8.425387165
18	153	209	362	183.2001	178.7999	362	4.978404156	5.100918924
19	137	167	304	153.8476	150.1524	304	1.844946805	1.890349553
20	107	200	307	155.3658	151.6342	307	15.05640713	15.42693394
Total	3081	3007	6088	3081	3007		χ^2=250.7309413	
							P=0.000	

Table 6: Chi-square value of mast cell density between Groups 1 and 2

Chi-square method								
Case number	Total observed values			Total expected values			$(OBS-EXP)^2/(EXP)$	
	Group 1	Group 2	Grand total	Group 1	Group 2	Grand total		
1	16	15	31	4.672346	26.32765	31	27.46280884	4.873800951
2	10	14	24	3.6173	20.3827	24	11.26222767	1.998697811
3	7	51	58	8.741809	49.25819	58	0.347056026	0.061591733
4	5	15	20	3.014417	16.98558	20	1.307895037	0.232111002
5	0	7	7	1.055046	5.944954	7	1.055045872	0.187238079
6	10	25	35	5.275229	29.72477	35	4.231751097	0.75100521
7	31	19	50	7.536042	42.46396	50	73.05656368	12.96528522
8	4	1	5	0.753604	4.246396	5	13.98490854	2.481889633
9	8	12	20	3.014417	16.98558	20	8.245721124	1.463361002
10	0	66	66	9.947575	56.05242	66	9.94757536	1.765387603
11	4	35	39	5.878113	33.12189	39	0.600074809	0.106494758
12	5	34	39	5.878113	33.12189	39	0.131178488	0.023280133
13	3	18	21	3.165138	17.83486	21	0.008615876	0.001529052
14	0	15	15	2.260813	12.73919	15	2.260812582	0.401224455
15	4	47	51	7.686763	43.31324	51	1.768263205	0.313812143
16	0	52	52	7.837484	44.16252	52	7.837483617	1.390911444
17	2	66	68	10.24902	57.75098	68	6.639298368	1.178270544
18	0	103	103	15.52425	87.47575	103	15.5242464	2.755074592
19	6	45	51	7.686763	43.31324	51	0.370138738	0.065688202
20	0	8	8	1.205767	6.794233	8	1.20576671	0.213986376
Total	115	648	763	115	648		χ^2=220.478072	
							P=0.000	

Algire and Chalkley were the first to suggest that tumor growth is closely related to the development of an intrinsic vascular network. Angiogenesis is necessary to provide oxygen, nutrients and immune cells to the tumor microenvironment and also removes its waste products.[17]

In the early phase of hyperplasia and dysplasia, infiltrating MCs degranulate and activate dermal fibroblasts which intensify angiogenesis. They also activate progelatinase B (matrix metalloproteinase family) which is involved in extracellular remodeling and angiogenic regulation. MCs activate and progressively intensify angiogenesis by releasing sequestered angiogenic activators.[18] As the neoplastic sequence progresses, angiogenic growth factor gene expression is upregulated in cancer cells. This is the progression to the second cancer phase where tumor cells directly control their angiogenic phenotype instead of manipulating inflammatory cells to indirectly affect neovascularization.[18]

Table 7: Chi-square value of mast cell density between Groups 2 and 3

Chi-square method								
Case number	Total observed values			Total expected values			$(OBS-EXP)^2/(EXP)$	
	Group 2	Group 3	Grand total	Group 2	Group 3	Grand total		
1	15	60	75	26.55738	48.44262	75	5.029599271	2.757343763
2	14	53	67	23.72459	43.27541	67	3.98606059	2.185251491
3	51	67	118	41.78361	76.21639	118	2.032900343	1.114483437
4	15	134	149	52.76066	96.23934	149	27.02519712	14.81584411
5	7	107	114	40.36721	73.63279	114	27.58106952	15.12058633
6	25	53	78	27.61967	50.38033	78	0.248470802	0.136217495
7	19	16	35	12.39344	22.60656	35	3.521749501	1.93070531
8	1	75	76	26.91148	49.08852	76	24.94863428	13.67742387
9	12	41	53	18.76721	34.23279	53	2.44016909	1.337757673
10	66	82	148	52.40656	95.59344	148	3.525926746	1.932995374
11	35	77	112	39.65902	72.34098	112	0.547326579	0.300057211
12	34	34	68	24.07869	43.92131	68	4.087947784	2.24110843
13	18	39	57	20.18361	36.81639	57	0.236238136	0.129511263
14	15	26	41	14.51803	26.48197	41	0.016000267	0.00877172
15	47	15	62	21.9541	40.0459	62	28.57312465	15.66445412
16	52	22	74	26.20328	47.79672	74	25.39647188	13.9229389
17	66	84	150	53.11475	96.88525	150	3.125865209	1.713672298
18	103	119	222	78.60984	143.3902	222	7.567502065	4.148681335
19	45	52	97	34.34754	62.65246	97	3.303726551	1.811180038
20	8	26	34	12.03934	21.96066	34	1.355248402	0.742978819
Total	648	1182	1830	648	1182		χ^2=270.2411918	
							P=0.000	

Table 8: Chi-square value of mast cell density between Groups 3 and 4

Chi-square method								
Case number	Total observed values			Total expected values			$(OBS-EXP)^2/(EXP)$	
	Group 3	Group 4	Grand total	Group 3	Group 4	Grand total		
1	60	182	242	61.38283	180.6172	242	0.031152457	0.010587178
2	53	90	143	36.27167	106.7283	143	7.71502573	2.621955265
3	67	76	143	36.27167	106.7283	143	26.03216037	8.847042427
4	134	78	212	53.77339	158.2266	212	119.6931939	40.67779045
5	107	93	200	50.72961	149.2704	200	62.41633116	21.21222065
6	53	151	204	51.74421	152.2558	204	0.030477201	0.010357692
7	16	224	240	60.87554	179.1245	240	33.08083823	11.24253904
8	75	75	150	38.04721	111.9528	150	35.88984989	12.19718303
9	41	85	126	31.95966	94.04034	126	2.557217956	0.869071772
10	82	251	333	84.46481	248.5352	333	0.071926677	0.024444316
11	77	218	295	74.82618	220.1738	295	0.063152927	0.021462553
12	34	248	282	71.52876	210.4712	282	19.69008788	6.691685991
13	39	318	357	90.55236	266.4476	357	29.34927217	9.974364494
14	26	299	325	82.43562	242.5644	325	38.63596073	13.13045014
15	15	417	432	109.576	322.424	432	81.62933566	27.74176962
16	22	65	87	22.06738	64.93262	87	0.000205748	6.99237E-05
17	84	148	232	58.84635	173.1536	232	10.75183066	3.654014907
18	119	62	181	45.9103	135.0897	181	116.3595998	39.54486686
19	52	221	273	69.24592	203.7541	273	4.295153268	1.459709938
20	26	177	203	51.49056	151.5094	203	12.61917855	4.288633997
Total	1182	3478	4660	1182	3478		χ^2=805.1321712	
							P=0.000	

Table 9: Correlation between microvessel density and mast cell density

Correlation coeficient between MVD and MCD, r = 0.109404487 (*P*=0.000)

MVD: Microvessel density, MCD: Mast cell density

Studies show that growth of vessels adjacent to tumor cells increases the chances of tumor cells entering blood circulation. Alternatively, primary tumors containing a high proportion of angiogenic cells will seed into the circulation giving rise to additional metastatic deposits, thus amplifying its growth and dissemination. This is because newly proliferating capillaries have fragmented permeable basement membranes, making them more accessible to tumor cells than mature vessels.[19]

A study by Rakesh *et al*. found that MCs and their regulatory role in angiogenesis and inflammation by the release of

Table 10: Correlation coefficient between microvessel density and mast cell density

Case number	MVD (x)	MCD (y)	x^2	y^2	x×y
1	90	16	8100	256	1440
2	61	10	3721	100	610
3	177	7	31,329	49	1239
4	95	5	9025	25	475
5	70	0	4900	0	0
6	144	10	20,736	100	1440
7	85	31	7225	961	2635
8	96	4	9216	16	384
9	98	8	9604	64	784
10	133	0	17,689	0	0
11	52	4	2704	16	208
12	53	5	2809	25	265
13	45	3	2025	9	135
14	54	0	2916	0	0
15	48	4	2304	16	192
16	56	0	3136	0	0
17	68	2	4624	4	136
18	64	0	4096	0	0
19	51	6	2601	36	306
20	55	0	3025	0	0
21	97	15	9409	225	1455
22	121	14	14641	196	1694
23	235	51	55,225	2601	11,985
24	73	15	5329	225	1095
25	133	7	17,689	49	931
26	51	25	2601	625	1275
27	81	19	6561	361	1539
28	107	1	11,449	1	107
29	143	12	20,449	144	1716
30	91	66	8281	4356	6006
31	113	35	12,769	1225	3955
32	64	34	4096	1156	2176
33	84	18	7056	324	1512
34	87	15	7569	225	1305
35	126	47	15,876	2209	5922
36	86	52	7396	2704	4472
37	150	66	22,500	4356	9900
38	112	103	12,544	10,609	11,536
39	110	45	12,100	2025	4950
40	160	8	25,600	64	1280
41	124	60	15,376	3600	7440
42	157	53	24,649	2809	8321
43	90	67	8100	4489	6030
44	181	134	32,761	17,956	24,254
45	195	107	38,025	11,449	20,865
46	129	53	16,641	2809	6837
47	115	16	13,225	256	1840
48	170	75	28,900	5625	12,750
49	219	41	47,961	1681	8979
50	125	82	15,625	6724	10,250
51	182	77	33,124	5929	14,014
52	158	34	24,964	1156	5372
53	207	39	42,849	1521	8073
54	112	26	12,544	676	2912
55	164	15	26,896	225	2460
56	157	22	24,649	484	3454
57	199	84	39,601	7056	16,716
58	153	119	23,409	14,161	18,207
59	137	52	18,769	2704	7124
60	107	26	11,449	676	2782
61	148	182	21,904	33,124	26,936
62	152	90	23,104	8100	13,680
63	193	76	37,249	5776	14,668
64	116	78	13,456	6084	9048
65	113	93	12,769	8649	10,509
66	98	151	9604	22,801	14,798
67	114	224	12,996	50,176	25,536
68	103	75	10,609	5625	7725
69	111	85	12,321	7225	9435
70	153	251	23,409	63,001	38,403
71	170	218	28,900	47,524	37,060
72	99	248	9801	61,504	24,552
73	152	318	23,104	101,124	48,336
74	189	299	35,721	89,401	56,511
75	160	417	25,600	173,889	66,720
76	238	65	56,644	4225	15,470
77	122	148	14,884	21,904	18,056
78	209	62	43,681	3844	12,958
79	167	221	27,889	48,841	36,907
80	200	177	40,000	31,329	35,400
Sum	9907	5423	1,414,087	921,489	796,448
Mean	123.8375	67.7875			
	Mean (x)=123.8375				
	Mean (y)=67.7875				
	Sum (x)=9907				
	Sum (y)=5423				
	Sum (x^2)=1,414,087				
	Sum (y^2)=921,489				
	Sum (x×y)=796,448				
Numerator	sum (x*y) - sum(x) x sum(y)/n = 124,877.2375				
Denominator	$\sqrt{(\text{Sum}(x^2)-\text{Sum}(x)/n)\times(\text{Sum}(y^2)-\text{Sum}(y)/n}$ = 1,141,427.024				
r (x, y)	0.109404487; (*P*=0.000)				

MVD: Microvessel density, MCD: Mast cell density

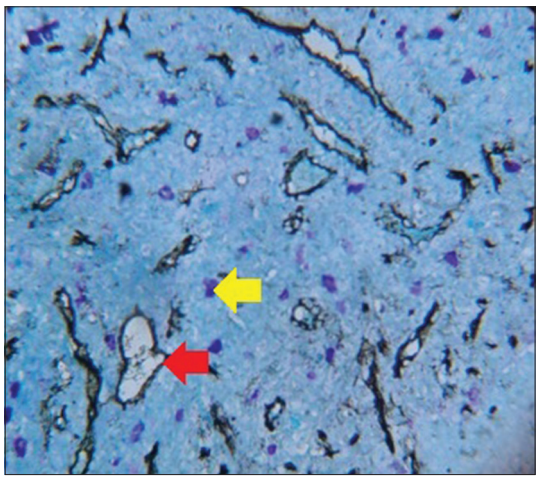

Figure 6: Photomicrograph of oral squamous cell carcinoma (red arrow - microvessel, yellow arrow - mast cell)

mediators may play an important role in tumor progression, facilitating the transformation of oral leukoplakia into invasive carcinoma,[20] reinforcing the observation of this study.

Another supporting study by Mohtasham *et al.* found a significant correlation between MCD and MVD in OSCC, concluding that MCs may promote tumor progression by regulating angiogenesis.[11,21]

Table 11: Mean or average values of microvessel density

Case number	Normal gingival tissue	Leukoplakia without dysplasia	Leukoplakia with dysplasia	OSCC
1	9	9.7	12.4	14.8
2	6.1	12.1	15.7	15.2
3	17.7	23.5	9	19.3
4	9.5	7.3	18.1	11.6
5	7	13.3	19.5	11.3
6	14.4	5.1	12.9	9.8
7	8.5	8.1	11.5	11.4
8	9.6	10.7	17	10.3
9	9.8	14.3	21.9	11.1
10	13.3	9.1	12.5	15.3
11	5.2	11.3	18.2	17
12	5.3	6.4	15.8	9.9
13	4.5	8.4	20.7	15.2
14	5.4	8.7	11.2	18.9
15	4.8	12.6	16.4	16
16	5.6	8.6	15.7	23.8
17	6.8	15	19.9	12.2
18	6.4	11.2	15.3	20.9
19	5.1	11	13.7	16.7
20	5.5	16	10.7	20

OSCC: Oral squamous cell carcinoma

Table 12: Mean or average values of mast cell density

Case number	Normal gingival tissue	Leukoplakia without dysplasia	Leukoplakia with dysplasia	OSCC
1	1.6	1.5	6	18.2
2	1	1.4	5.3	9
3	0.7	5.1	6.7	7.6
4	0.5	1.5	13.4	7.8
5	0	0.7	10.7	9.3
6	1	2.5	5.3	15.1
7	3.1	1.9	1.6	22.4
8	0.4	0.1	7.5	7.5
9	0.8	1.2	4.1	8.5
10	0	6.6	8.2	25.1
11	0.4	3.5	7.7	21.8
12	0.5	3.4	3.4	24.8
13	0.3	1.8	3.9	31.8
14	0	1.5	2.6	29.9
15	0.4	4.7	1.5	41.7
16	0	5.2	2.2	6.5
17	0.2	6.6	8.4	14.8
18	0.3	10.3	11.9	6.2
19	0.6	4.5	5.2	22.1
20	0	0.8	2.6	17.7

OSCC: Oral squamous cell carcinoma

A pioneering study by Tomita *et al*. put forth two reasons for the conflicting reports on the role of MCs.[10] First, MC's cytotoxic function suppresses tumor activities initially when they infiltrate tumor tissue. However, after infiltration, tumor cells might instigate MC's angiogenic properties while suppressing their cytotoxic functions, thereby leading to tumor angiogenesis. Second, cell-mediated cytotoxic effects of MCs are reported when MC–tumor ratio is >20:1. Conversely, these effects are nullified when the MC–tumor ratio is increased from 10:1 to 1:100 leading to tumor progression. Hence, the effect of MCs against cancer cells might depend on the concentration of MC products in the microenvironment. Based on these findings, the researcher hypothesized that reversing this process, that is, enhancing the cytotoxic functions of MCs and suppressing their angiogenic functions, could lead to a new anticancer treatment strategy.[10,15]

CONCLUSION

In this study, the MVD and MCD increased significantly in the following cases; the increase was greater in cases of OSCC, followed by leukoplakia with dysplasia, leukoplakia without dysplasia and normal gingival tissue. Therefore, it is concluded that MCs may play a significant role in angiogenesis by releasing pro-angiogenic and angiogenic factors which may in turn favor the progression of premalignant lesion to a malignant one. A limitation of

this study is that the role of MCs in angiogenesis and the role of angiogenesis in the evolution of squamous cell carcinoma from epithelial dysplasia need to be validated using a larger sample size and further follow-up studies. A specially stratified sample using differently graded cases of OSCC and leukoplakia will lead to even more conclusive results. Immunostaining of MCs with tryptase will render a specialized understanding of human MCs – thereby advancing cancer research and developing better treatment.

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Conflicts of interest

There are no conflicts of interest.

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